

Sterile Fecal Filtrate from a Healthy Donor Improves Microbial Diversity in Patients with Hepatic Encephalopathy

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ABSTRACT

Background & Aims: Hepatic encephalopathy (HE) remains one of the most debilitating complications of liver cirrhosis. Changes in gut microbiome composition have been linked to liver diseases and its complications including HE. Recent randomized controlled trials showed fecal microbiota transplantation to be safe and effective in HE treatment. However, transferring unidentified live bacteria could cause various complications, including infections, especially in immunocompromised patients. This study aimed to evaluate the safety and efficacy of sterile fecal filtrate transfer (SFFT) for the modulation of the intestinal microbiome of patients with cirrhosis and HE.

Methods: A custom-made air pressure filtration device was used for the sterile fecal filtrate preparation. Seven patients received SFFT from the same healthy donor. Patients were monitored at least 30 days after the procedure. Cognition tests, blood and stool sampling were performed to assess the safety and efficacy of SFFT on HE, liver function, and stool microbiome composition on follow-up days 7 and 30.

Results: SFFT was well tolerated and resulted in fluctuations in the microbial composition of study participants: α -diversity increased in 4/7 of the patients, without robust engraftment of donors' microbial composition as assessed by β -diversity analysis. No significant effect on cognition tests or liver function was noted after the procedure. One death occurred three months after the procedure; however, it was not related to the SFFT.

Conclusions: Despite the effect on the gut microbiome, we did not observe robust improvement in patients' liver function or HE cognition tests after the procedure.

Key words: fecal microbiota transplantation – FMT – encephalopathy – cirrhosis – fecal-filtrate – microbiome-modulation.

Abbreviations: CTP: Child-Turcotte-Pugh; DST: digital symbol test; FMT: fecal microbiota transplantation; HCV: hepatitis C virus; HE: Hepatic encephalopathy; MELD: Model of End-Stage Liver Disease; NAD: non-absorbable disaccharides; NCT: number connection test; rCDI: recurrent *Clostridioides difficile* infection; SFF: sterile fecal filtrate; SFFT: SFF transfer.

INTRODUCTION

Hepatic encephalopathy (HE) is a disorder characterized by marked cognitive and psychomotor dysfunction and remains one of the most severe complications of liver cirrhosis [1]. Hepatic encephalopathy can manifest in a range of clinical symptoms starting from subtle cognitive deficits and progressing to severe encephalopathy or even coma, as defined by West Haven criteria [2]. Prevalence of

minimal hepatic encephalopathy is up to 80% and clinically apparent form (overt HE) is diagnosed in 30-45% of patients with liver cirrhosis [3]. Hepatic encephalopathy significantly decreases patients and their relatives quality of life [4] and also was found to be a prognostic factor, as the one-year survival rate after the first episode of overt HE can be as low as 40% [5]. Even though current standard of care treatment is partly effective in HE, patients experience concurrent breakthrough episodes, making HE therapeutics one of the most important unmet goals in liver cirrhosis [6].

Disruption of equilibrium between host and gut microbiome is established in liver cirrhosis and HE [7]. Studies show decreased abundance of short-chain fatty acid-producing, as well as an increase in potentially pathogenic taxa, while gut-derived ammonia production is considered a key element in HE

pathogenesis. Specific gut and sigmoid mucosal microbiome profiles exist for cirrhosis and specific taxa have been linked to systemic inflammation, and poor cognition when comparing patients with and without HE [8]. Furthermore, microbial compositional changes become more apparent with the decompensation of stable disease [9].

Currently used standard-of-care treatments include drugs directed at gut microbiota. First choice of drugs being non-absorbable disaccharides (NAD), such as lactulose [10, 11]. It has been shown that NAD decreases colonic pH [12], promotes usage of ammonia as the substrate for amino acid synthesis, and inhibits ammonia-producing bacteria [13] leading to reduced ammonia concentrations [11]. The poorly absorbed antibiotic, rifaximin has also shown a profound efficacy in treating acute and chronic hepatic encephalopathy through gut microbiome modulation. Decreased pathogen abundance, reduced gut-derived systemic inflammation and improved cognition have been shown to be properties of this drug [6]. Nevertheless, even concomitant therapy with lactulose and rifaximin cannot prevent breakthrough episodes of HE of patients in around 20% indicating the need for alternative HE therapies [6].

At least two randomized controlled trials [14, 15] and an open-label study [16] have shown safety and efficacy of fecal microbiota transplantation (FMT) in patients with cirrhosis and HE encephalopathy. Fecal microbiota transplantation was shown to improve cognition, with extending effects in possible reduction of hospitalizations [14-16]. While FMT appears to be a generally safe and well-tolerated procedure, a possible transfer of pathogenic, including ESBL bacteria [17] is a known complication of FMT and the following event could be detrimental for an immunocompromised patient such as in cirrhosis [18].

In 2017 Ott et al. [19], showed sterile fecal filtrate (SFF) to be effective in the treatment of recurrent *Clostridioides difficile* infection while reducing potential bacteria transfer risks [19]. In this study, using the same methodology proposed by Ott et al. [19], we aimed to evaluate the safety of SFF and the effect on microbial parameters in patients with hepatic encephalopathy using an open-label trial.

METHODS

Study Patients

Included patients were at least 18 years old and had been diagnosed with at least one episode of overt HE associated with hepatitis C virus (HCV) or alcohol-induced liver cirrhosis, were taking lactulose daily, and were not recently on additional antibiotics or consuming alcohol in the past 3 months). Only patients with Child-Turcotte-Pugh (CTP) score B and C were enrolled in the study. None of the patients were on probiotic therapy at the time of inclusion in the study. None of the patients were on rifaximin therapy due to the reimbursement policy.

Exclusion criteria: recent antibiotic exposure (1 month), active alcohol intake (in the past 3 months), active infection, gastrointestinal bleeding (over the last 6 weeks), history of transjugular intrahepatic portosystemic shunt placement, hepatocellular carcinoma.

Donor Selection

One donor, unrelated to the study participants provided the stool donations for the preparation of sterile fecal filtrate [20, 21]. The selected donor was a physically and mentally healthy adult with a normal body mass index. Rigorous screening of the donor was performed following recommendations previously published in the European consensus of fecal microbiota transplantation [20]. In short, donors' blood was screened for the following infections: hepatitis A, B, and C viruses, human immunodeficiency virus, Epstein Barr virus, Cytomegalovirus, *Treponema pallidum*. Stool samples were assayed for pathogenic agents: *Clostridioides difficile*, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, norovirus, *Giardia lamblia*, and *Cryptosporidium parvum*, protozoa and helminths.

Sterile Fecal Filtrate Preparation and Administration of the Filtrate

50g of fresh feces from the thoroughly screened donor were collected and stored at 4°C until further processing, which was performed within 4 hours. Stool slurry was made with a standard commercial blender using donor stool material and approximately 500ml of sterile normal saline (0.9% sodium chloride). The prefiltered slurry was then filtered using a custom-built air pressure filtration system using consecutive filters to remove stool debris (first with a retention rating of 6-15 µm and second – with 0.4-0.8 µm [Seitz K 700 P 60 D and Seitz KS 50 P 60 D; PALL]). In the last step, the fecal filtrate was depleted of microbes by using a 0.2 µm sterilizing-grade membrane filter (SUPOR EKV Filter Mini Kleenpak, PALL). The precise methodology is described by Ott et al. [19].

The sterile fecal filtrate was administered via a commercially available nasojejunal tube, which was placed in patients' descending part of the duodenum by gastroscopy. Abdominal X-rays were performed for all the patients following the endoscopy to confirm the correct placement of the nasojejunal tube.

Study Design and Procedures

The study protocol was approved by the Regional Ethics Committee (2020-02-18, No. BE-2-22, Kaunas, Lithuania). All participants provided written informed consent before enrollment in the study. Seven patients were enrolled in this open-label pilot study of sterile fecal filtrate for the treatment of hepatic encephalopathy. Included patients underwent a SFF transfer (SFFT) procedure. Cognitive testing [number connection test (NCT) A, NCT B, digit symbol test (DST)], stool and serum collection for further biochemical and sequencing analysis were performed before the SFFT and on consecutive days 7 and 30 after the procedure. Safety was assessed on days 1, 7, and 30 after the procedure during the control visits and via telephone call 3 months after the procedure. A schematic of the study is shown in Fig. 1.

Isolation of Nucleic Acids, Sequencing, and Statistical Analysis

Nucleic acids from stool samples were extracted using QIAamp Fast DNA Stool Mini Kit (Qiagen) according to the manufacturer's protocol.

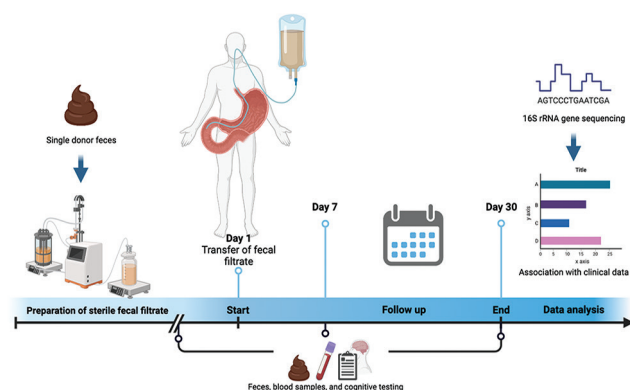


Fig. 1. Workflow of the study.

Isolated DNA from stool samples was amplified using specific primer pair set 27F (AGAGTTTGATCCTGGCTCAG) and 338R (TGCTGCCTCCGTTAGGAGT), dual-indexing

was used in the process of PCR. The PCR products were purified and normalized utilizing the Invitrogen SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, Waltham, USA). 16S rRNA gene sequencing was performed on Illumina MiSeq platform according to the manufacturer's instructions with MiSeq Reagent Kit v3. The acquired sequencing data were assigned into amplicon sequencing variants and taxonomically annotated against the RDP v16 database using the 'dada2' software package in R (V.1.10) following the DADA2 workflow (<http://benjjneb.github.io/dada2/tutorial.html>). Rarefaction was used to normalize acquired data. All the samples were rarefied to 3800 reads per sample prior to α -diversity, β -diversity, and compositional analyses. Shannon index was used as a measure of alpha diversity. Bray Curtis dissimilarity on relative abundances was used as a measure of β -diversity. Nonparametric tests were used for statistical analyses where appropriate.

Table I. Demographic and clinical characteristics of subject groups

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Gender	Male	Male	Male	Male	Male	Male	Male
Age	52	60	42	56	58	69	43
Etiology	HCV	Alcohol	Alcohol	Alcohol	Alcohol	Alcohol	HCV
Esophageal varices	F1	F0	F2	F1	F1	F2	F2
Ascites	None	Slight	Slight	None	Slight	None	Slight
CTP score	8	12	9	8	9	8	8
MELD before	13	17	13	13	17	15	14
MELD 30 days	13	18	13	13	15	14	14
Time of last HE episode before enrollment (days)	58	45	42	67	54	82	74
No. of past HE episodes (1-year preceding enrollment)	1	3	2	1	2	2	1
Biochemical tests							
Hemoglobin, g/l (day 0/30)	141/135	86/94	99/108	154/158	108/112	142/145	106/110
Leukocytes, $\times 10^9/l$ (day 0/30)	9.4/6.4	2.9/2.9	2.9/3.1	8.4/6.9	9.9/9.4	4.1/3.8	4.7/3.3
Platelets $\times 10^9/l$ (day 0/30)	114/101	59/71	130/110	165/141	135/144	93/87	132/112
AST, IU/l (day 0/30)	59/48	48/46	22/25	44/33	148/62	54/50	56/60
ALT, IU/l (day 0/30)	76/60	31/35	8/14	77/49	66/27	52/47	34/30
ALP, IU/l (day 0/30)	116/130	147/137	61/59	63/66	189/108	129/103	117/159
GGT, IU/l (day 0/30)	122/147	46/56	10/22	52/42	145/112	43/40	208/142
Bilirubin, $\mu\text{mol/l}$ (day 0/7/30)	63.4/57.2/57.2	47/67.6/72	28/14/29	49/46/45	62.7/42.8/48.8	23.7/21.3/23.4	33/42.1/41.8
Albumin, g/l (day 0/7/30)	39/36/33	26/26/26	31/29/26	39/40/42	40/38/37	34/36/38	29/31/35
INR (day 0/7/30)	1.2/1.1/1.2	1.8/1.8/1.5	1.5/1.4/1.3	1.3/1.3/1.2	1.3/1.1/1.2	1.9/1.8/1.6	1.6/1.6/1.5
Ammonia, $\mu\text{mol/l}$ (day 0/7/30)	79/74/64	65/52/75	47/43/47	31/29/12	73/64/72	37/54/52	55/57/48
Creatinine, $\mu\text{mol/l}$ (day 0/7/30)	76/68/78	69/71/69	75/69/89	71/72/84	132/124/118	74/82/82	57/63/66
CRP, mg/l (day 0/7/30)	5.5/5/4.7	9.8/6.2/5.4	5.8/3.1/5.3	3.3/1/2.31	19/5/9.47	1/1/1	5.2/3.4/1.4
Psychometric tests							
NCT-A, s (day 0/7/30)	81/80/88	120/120/120	83/73/68	90/88/84	98/79/72	120/120/120	61/70/53
NCT-B, s (day 0/7/30)	77/85/74	120/120/120	103/65/65	70/58/56	89/87/56	120/120/120	63/63/50
DST, symbols correct (day 0/7/30)	31/27/29	17/21/18	34/35/32	30/31/27	23/25/25	20/20/21	22/18/22
Adverse events							
Day 0	Nausea/ bloating	-	-	Nausea	Nausea	Bloating	-

MELD: Model of End-Stage Liver Disease; CTP: Child-Turcotte-Pugh; HCV: hepatitis C virus; NCT: number connection test; DST: digital symbol test. All the included patients were on a regular diet. None of the patients were active drinkers for at least three months prior to the inclusion in the study.

RESULTS

Patients

Seven patients with cirrhosis and a history of overt HE were included in the study. All the patients were male, the median age was 56 years (range 43-69), five had alcohol-associated cirrhosis, while two had HCV (eradicated in both patients) induced cirrhosis. Median Model of End-Stage Liver Disease (MELD) at the time of inclusion was 14 (range 13-17). Median Child-Turcotte-Pugh score was 8 (range 8-12). All patients were on lactulose treatment and were on a regular diet. Patient characteristics are presented in Table I.

Safety

Five minor side effects including nausea and bloating were registered during the study, all of which were related to the SFF administration. None of the patients developed infections during one month of follow-up. However, Patient 2 developed pneumonia three months after the SFF transfer procedure and died due to complications related to infection. This event, however, was not registered as related to the procedure because of the extended timeline to the event.

Cognitive Performance and Liver Function after SFFT

The cognitive effect of SFFT procedure was measured using three standard tests, all of which are used for diagnosis of HE: NCT A and B and DST. There were no significant differences in cognitive tests at baseline and after SFFT procedure, however, a tendency towards better scores in NCT B was noted ($p=0.06$, Wilcoxon paired test) (Fig. 2). No significant alterations in liver function tests were noted as well. Levels of albumin, bilirubin, INR, and ammonia remained rather stable through the observational period (Fig. 3). Median MELD score did not change significantly ($p=0.441$); however, there was an increase from 17 to 18 in patient 2, and a decrease from 17 to 15 and from 15 to 14 in patients 5 and 6, respectively. None of the study participants experienced breakthrough episodes of HE 30 days after the procedure.

Microbiome Composition after SFFT

When comparing patients' samples at baseline, day 7, and day 30 after the SFFT procedure, we observed significant bacterial community shifts in all patients (Fig. 4). However, β -diversity analysis did not reveal the 'engraftment' of donors' microbiota, as most samples clustered near the samples of the same individual and not the donor (Fig. 5). Dynamics in α -diversity, when compared at baseline and 30 days after the procedure, were not statistically significant as well ($p=0.56$, Wilcoxon paired test). However, there was a robust increase in α -diversity for patients 1, 5, 6, and 7 (Fig. 6).

For patients 5 and 6 increase in α -diversity was also associated with a decrease in MELD score. While a deceased patient (patient 2) had a gradual decrease in α -diversity through the observation period.

DISCUSSION

Hepatic encephalopathy remains an underserved burden in liver cirrhosis. Currently approved therapies targeting gut

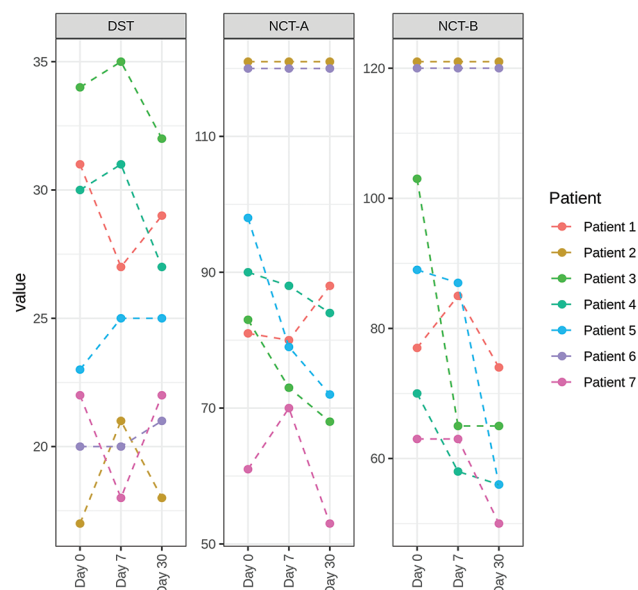


Fig. 2. Psychometric hepatic encephalopathy tests reflecting the severity of hepatic encephalopathy. DST: digit symbol test. Values represent a number of correctly drawn symbols within 90 seconds. A higher number indicates a better score. NCT-A and NCT-B: number connection test A and B respectively. Values represent the time needed to connect 25 numbers in ascending order. Higher values indicate a longer time needed to complete the test. The maximum time for the test – 120s. No statistical significance was found when comparing baseline values vs either day 7 or day 30 (Wilcoxon paired test).

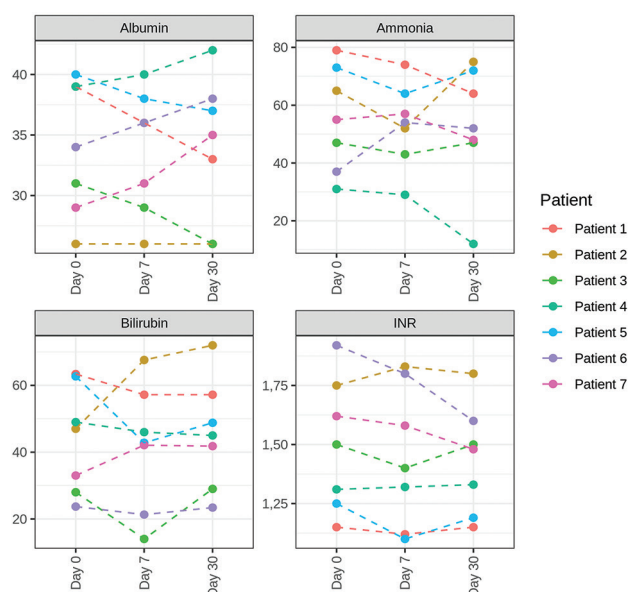


Fig. 3. Dynamics of biochemical tests representing the liver function. Day 0 represents the day before SFFT procedure, while days 7 and 30 – represent values on respective days after the procedure. No statistical significance was found when comparing baseline values vs day 30 (Wilcoxon paired test).

microbiome milieu [22] are proven to be effective. However, a portion of these patients still suffer from exacerbation of this condition, indicating a need for a novel look in therapeutics [6]. Evidence suggests that changes in gut microbiome

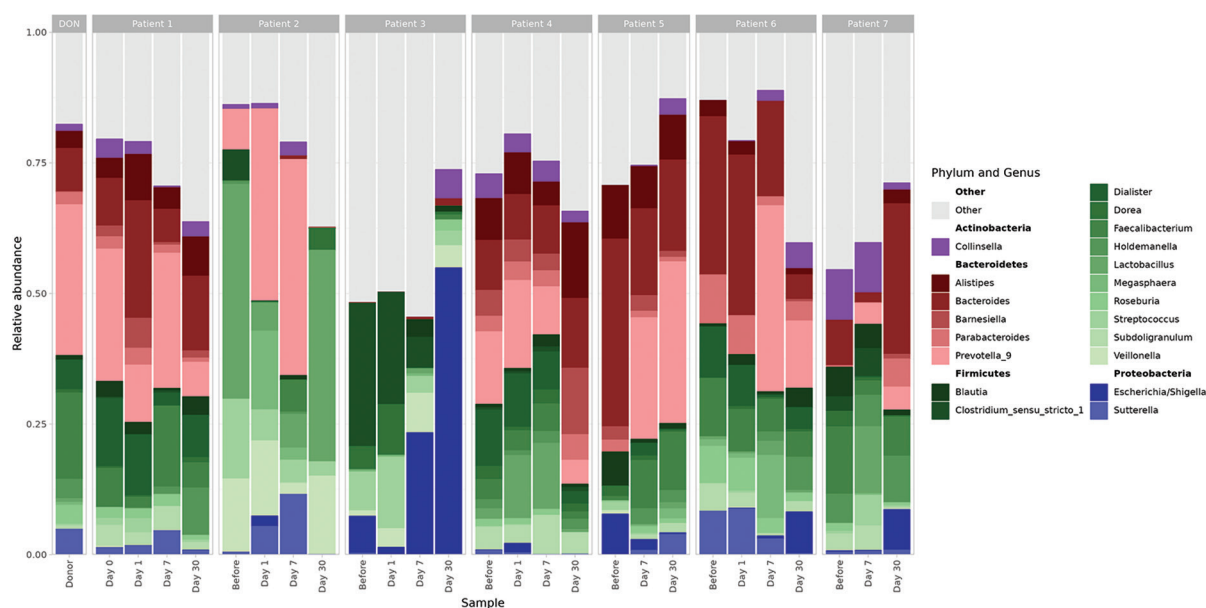


Fig. 4. Relative abundances of 20 most abundant taxa across the study cohort (donor stool sample and patients at baseline, day 1, 7, and 30 after SFFT procedure). Taxa are displayed at Phylum and Genus levels. Bacterial phyla and genera not in the 20 most abundant taxa are represented as other.

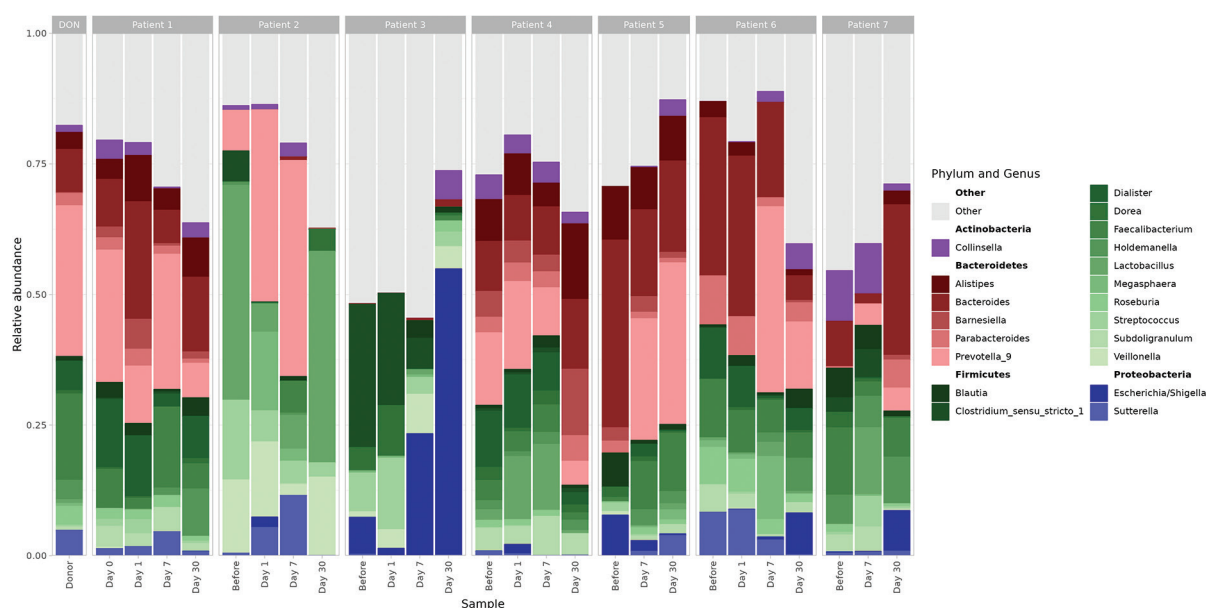


Fig. 5. Non-multidimensional scaling graph on Bray-Curtis dissimilarity representing β -diversity. Distances between samples denote dissimilarities between samples based on the samples' bacterial community structure. The closer the samples – the more similar the microbial composition between samples.

play a pivotal role in the gut-liver-brain axis in cirrhosis [23], making modulation of gastrointestinal microbiome a suitable target in the treatment of complications related to liver cirrhosis.

Recently published studies aimed to restore a disturbed gut microbiome in patients with cirrhosis via FMT and showed promising results regarding cognition scores in HE and outcomes in cirrhosis [14-16]. Fecal microbiota transplantation is shown to be a safe procedure even in cirrhotic patients [24]; however, a transfer of infectious agents is possible and transfer of ESBL bacteria has been previously reported [17], even in one of the FMT for HE trials [16].

Transferring an entire donor's gut microbiome community shows the highest success rates in dysbiosis-related recurrent *Clostridioides difficile* infection (rCDI). It was shown that autologous stool transplantation could achieve clinical effect in up to 60% [25], suggesting alternative mechanisms of microbiome restoration. Moreover, a recent study by Ott et al. [19] showed that usage of fecal filtrate without viable bacteria could also restore the gut microbiome and alleviate symptoms in rCDI patients. In our study we have used the proposed custom-made air pressure filtration device for SFF preparation following the previously described methodology and administered the filtrate to patients with cirrhosis [19].

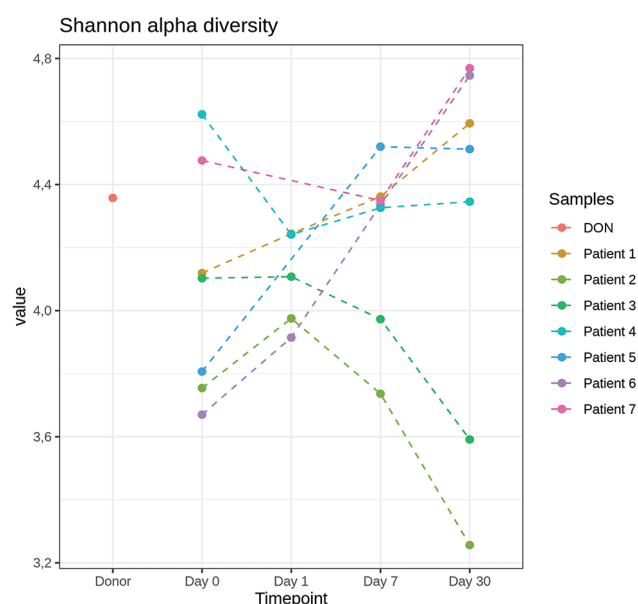


Fig. 6. Dynamics of α -diversity (assessed by Shannon diversity index) at baseline and days 0, 1, 7, 30 after the procedure. No statistical significance was found when comparing baseline α -diversity values vs values on day 30 (Wilcoxon paired test).

Although we did not see clear donor microbiome engraftment signals after the procedure, we did observe fluctuations in the most prevalent taxa, and an increase in α -diversity in 4/7 patients after the procedure. Moreover, this increase was associated with a decrease in MELD score for 2 patients. While the exact mechanisms of how SFF affects the gut microbiome are unknown, we could postulate that the fecal filtrate contains bacterial wall components, small DNA fragments, bacteriocins, and metabolites which could affect intestinal bacteria directly or modulate patients' innate immune system through interaction with pattern recognition receptors [26]. As the gut microbiome contains not only bacteria but also viruses – a possible viral specifically transfer of bacteriophages could impact the shape of the microbiome after the procedure [27]. While we did not perform viral analysis in our study, it was shown that phageome tends to resemble donor composition more than 6 weeks after the SFFT procedure [19]. Despite noted effects on microbiome composition, we could not establish significant associations between SFFT and patients' cognition, as we did not see significant dynamics in DST and NCT A. However, we did see a tendency towards improvement in NCT B test scores, indicating a need for future higher scale clinical studies.

We must address several limitations of our study. First, due to a small sample size, our study is underpowered making interpretations of statistical significance difficult. Second, even though we have not observed robust cognitive improvements, two patients still benefited from a decrease in MELD score. Even though this could be due to natural fluctuations of liver disease, this was associated with increases in α -diversity after SFFT. However, the lack of virome, metabolome, and inflammatory markers analysis makes it difficult to postulate of possible positive SFFT effect on patients' disease. Third, a second arm of clinically matched

patients on standard of care treatment could help to elucidate the putative effects of SFFT. Lastly, we excluded patients with recent exposure (one month before enrollment in the study) to antibiotics from the study. We must admit that residual effects of antibiotics on gut microbiome composition could persist for a longer period.

CONCLUSIONS

Our case series study shows that the SFFT procedure is safe in patients with recurrent HE and was associated with a significant increase in α -diversity of the gut microbiome in a proportion of patients. However, we did not observe robust improvement in patients' liver function or HE cognition tests after the sterile fecal filtrate transfer procedure.

Conflicts of interest: None to declare.

Authors' contribution: R.G., J.S.B., J.S., S.S. and J.K. conceived the study. J.S., J.B., A.F., detailed the methodology. R.G., J.S., I.V., E.K., collected and analysed the data. R.G. and J.K. drafted the manuscript. J.S.B., J.S., A.F., S.S., J.K. revised the manuscript. J.S., C.B., A.F., J.K. provided the resources and the funding. J.S.B., S.S., J.K. supervised the study process. All authors have had full access to the study data, proofread and approved the manuscript.

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REFERENCES

1. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy - Definition, nomenclature, diagnosis, and quantification: Final report of the Working Party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002;35:716–721. doi:10.1053/jhep.2002.31250
2. Weissenborn K. Hepatic Encephalopathy: Definition, Clinical Grading and Diagnostic Principles. *Drugs* 2019;79(Suppl 1):5–9. doi:10.1007/s40265-018-1018-z
3. Poordad FF. Review article: the burden of hepatic encephalopathy. *Aliment Pharmacol Ther* 2007;25 Suppl 1:3–9. doi:10.1111/j.1746-6342.2006.03215.x
4. Fabrellas N, Moreira R, Carol M, et al. Psychological Burden of Hepatic Encephalopathy on Patients and Caregivers. *Clin Transl Gastroenterol* 2020;11:e00159. doi:10.14309/ctg.0000000000000159
5. Bustamante J, Rimola A, Ventura PJ, et al. Prognostic significance of hepatic encephalopathy in patients with cirrhosis. *J Hepatol* 1999;30:890–895. doi:10.1016/S0168-8278(99)80144-5
6. Bass NM, Mullen KD, Sanyal A, et al. Rifaximin treatment in hepatic encephalopathy. *N Engl J Med* 2010;362:1071–1081. doi:10.1056/NEJMoa0907893
7. Bajaj JS, Khoruts A. Microbiota changes and intestinal microbiota transplantation in liver diseases and cirrhosis. *J Hepatol* 2020;72:1003–1027. doi:10.1016/j.jhep.2020.01.017
8. Bajaj JS, Hylemon PB, Ridlon JM, et al. Colonic mucosal microbiome differs from stool microbiome in cirrhosis and hepatic encephalopathy

- and is linked to cognition and inflammation. *Am J Physiol Gastrointest Liver Physiol* 2012;303:G675–G685. doi:[10.1152/ajpgi.00152.2012](https://doi.org/10.1152/ajpgi.00152.2012)
9. Bajaj JS, Heuman DM, Hylemon PB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940–947. doi:[10.1016/j.jhep.2013.12.019](https://doi.org/10.1016/j.jhep.2013.12.019)
 10. Bajaj JS, Ridlon JM, Hylemon PB, et al. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G168–G175. doi:[10.1152/ajpgi.00190.2011](https://doi.org/10.1152/ajpgi.00190.2011)
 11. Wang JY, Bajaj JS, Wang JB, et al. Lactulose improves cognition, quality of life, and gut microbiota in minimal hepatic encephalopathy: A multicenter, randomized controlled trial. *J Dig Dis* 2019;20:547–556. doi:[10.1111/1751-2980.12816](https://doi.org/10.1111/1751-2980.12816)
 12. Avery GS, Davies EF, Brogden RN. Lactulose: a review of its therapeutic and pharmacological properties with particular reference to ammonia metabolism and its mode of action of portal systemic encephalopathy. *Drugs* 1972;4:7–48. doi:[10.2165/00003495-197204010-00003](https://doi.org/10.2165/00003495-197204010-00003)
 13. Vince AJ, Burridge SM. Ammonia production by intestinal bacteria: the effects of lactose, lactulose and glucose. *J Med Microbiol* 1980;13:177–191. doi:[10.1099/00222615-13-2-177](https://doi.org/10.1099/00222615-13-2-177)
 14. Bajaj JS, Kassam Z, Fagan A, et al. Fecal microbiota transplant from a rational stool donor improves hepatic encephalopathy: A randomized clinical trial. *Hepatology* 2017;66:1727–1738. doi:[10.1002/hep.29306](https://doi.org/10.1002/hep.29306)
 15. Bajaj JS, Salzman NH, Acharya C, et al. Fecal Microbial Transplant Capsules Are Safe in Hepatic Encephalopathy: A Phase I, Randomized, Placebo-Controlled Trial. *Hepatology* 2019;70:1690–1703. doi:[10.1002/hep.30690](https://doi.org/10.1002/hep.30690)
 16. Bloom PP, Donlan J, Torres Soto M, Daidone M, Hohmann E, Chung RT. Fecal microbiota transplant improves cognition in hepatic encephalopathy and its effect varies by donor and recipient. *Hepatol Commun* 2022;6:2079–2089. doi:[10.1002/hep4.1950](https://doi.org/10.1002/hep4.1950)
 17. DeFilipp Z, Bloom PP, Torres Soto M, et al. Drug-Resistant *E. coli* Bacteremia Transmitted by Fecal Microbiota Transplant. *N Engl J Med* 2019;381:2043–2050. doi:[10.1056/NEJMoa1910437](https://doi.org/10.1056/NEJMoa1910437)
 18. Arvaniti V, D'Amico G, Fede G, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. *Gastroenterology* 2010;139:1246–1256.e5. doi:[10.1053/j.gastro.2010.06.019](https://doi.org/10.1053/j.gastro.2010.06.019)
 19. Ott SJ, Waetzig GH, Rehman A, et al. Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With *Clostridium difficile* Infection. *Gastroenterology* 2017;152:799–811.e7. doi:[10.1053/j.gastro.2016.11.010](https://doi.org/10.1053/j.gastro.2016.11.010)
 20. Cammarota G, Ianiro G, Tilg H, et al. European consensus conference on faecal microbiota transplantation in clinical practice. *Gut* 2017;66:569–580. doi:[10.1136/gutjnl-2016-313017](https://doi.org/10.1136/gutjnl-2016-313017)
 21. van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* 2013;368:407–415. doi:[10.1056/NEJMoa1205037](https://doi.org/10.1056/NEJMoa1205037)
 22. Bajaj JS, Heuman DM, Sanyal AJ, et al. Modulation of the metabiome by rifaximin in patients with cirrhosis and minimal hepatic encephalopathy. *PLoS One* 2013;8:e60042. doi:[10.1371/journal.pone.0060042](https://doi.org/10.1371/journal.pone.0060042)
 23. Bajaj JS. The role of microbiota in hepatic encephalopathy. *Gut Microbes* 2014;5:397–403. doi:[10.4161/gmic.28684](https://doi.org/10.4161/gmic.28684)
 24. Bajaj JS, Fagan A, Gavis EA, Kassam Z, Sikaroodi M, Gillevet PM. Long-term Outcomes of Fecal Microbiota Transplantation in Patients With Cirrhosis. *Gastroenterology* 2019;156:1921–1923.e3. doi:[10.1053/j.gastro.2019.01.033](https://doi.org/10.1053/j.gastro.2019.01.033)
 25. Kelly CR, Khoruts A, Staley C, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent *Clostridium difficile* Infection: A Randomized Trial. *Ann Intern Med* 2016;165:609–616. doi:[10.7326/M16-0271](https://doi.org/10.7326/M16-0271)
 26. Dziarski R. Recognition of bacterial peptidoglycan by the innate immune system. *Cell Mol Life Sci* 2003;60:1793–1804. doi:[10.1007/s00018-003-3019-6](https://doi.org/10.1007/s00018-003-3019-6)
 27. Shkoporov AN, Hill C. Bacteriophages of the Human Gut: The “Known Unknown” of the Microbiome. *Cell Host Microbe* 2019;25:195–209. doi:[10.1016/j.chom.2019.01.017](https://doi.org/10.1016/j.chom.2019.01.017)